We claim:

1. A protein having the polypeptide sequence depicted in SEQ ID NO:2 or a polypeptide sequence obtainable from SEQ ID NO:2 by substitution, insertion or deletion of up to 15% of the amino acids, and having the enzymatic activity of a phosphoribosyl-pyrophosphate synthetase.

2. A protein as claimed in claim 1, which is no longer subject to feedback inhibition by secondary products of metabolic pathways starting from products of the enzyme.

3. A protein as claimed in claim 1, which is no longer inhibited by intermediates of purine biosynthesis, in particular by purine bases, purine nucleosides, purine nucleotide 5'-monophosphates or purine nucleotide 5'-diphosphates or purine nucleotide 5'-triphosphates.

4. A protein as claimed in claim 1, in which one or more of the following amino acid substitutions are present: lysine at position 7 replaced by valine, aspartate at position 52 replaced by histidine, leucine at position 133 replaced by isoleucine, aspartate at position 186 replaced by histidine, alanine at position 193 replaced by valine or histidine at position 196 replaced by glutamine.

5. A nucleic acid sequence coding for a protein as claimed in claim 1.

6. A protein having the polypeptide sequence depicted in SEQ ID NO:13 or a polypeptide sequence obtainable from SEQ ID NO:13 by substitution, insertion or deletion of up to 10% of the amino acids, and having the enzymatic activity of a phosphoribosyl-pyrophosphate synthetase.

7. A nucleic acid sequence coding for a protein as claimed in claim 6.

8. A protein having the polypeptide sequence depicted in SEQ ID NO:5 or a polypeptide sequence obtainable from SEQ ID NO:5 by substitution, insertion or deletion of up to 10% of the amino acids, and having the enzymatic activity of a glutamine-phosphoribosyl-pyrophosphate amidotransferase.

9. A protein as claimed in claim 8, which is no longer subject to feedback inhibition by secondary products of metabolic pathways starting from products of the enzyme.

10. A protein as claimed in claim 8, which is no longer inhibited by intermediates of purine biosynthesis, in particular by purine bases, purine nucleosides, purine nucleotide 5'-monophosphates or purine nucleotide 5'-diphosphates or purine nucleotide 5'-triphosphates.

11. A protein as claimed in claim 8, in which one or more of the following amino acid substitutions are present: aspartate at position 310 replaced by valine, lysine at position 333 replaced by alanine or alanine at position 417 replaced by tryptophan.

12. A nucleic acid sequence coding for a protein as claimed in claim 8.

13. A protein having the polypeptide sequence depicted in SEQ ID NO:8 and 9 or a polypeptide sequence obtainable from SEQ ID NO:8 and 9 by substitution, insertion or deletion of up to 20% of the amino acids, and having the enzymatic activity of an IMP dehydrogenase.

14. A protein as claimed in claim 13, which is no longer subject to feedback inhibition by secondary products of metabolic pathways starting from products of the enzyme.

15. A protein as claimed in claim 13, which is no longer inhibited by intermediates of purine biosynthesis, in particular by purine bases, purine nucleosides, purine nucleotide 5'-monophosphates or purine nucleotide 5'-diphosphates or purine nucleotide 5'-triphosphates.

16. A nucleic acid sequence coding for a protein as claimed in claim 13.

17. A protein having the polypeptide sequence depicted in SEQ ID NO:11 or a polypeptide sequence obtainable from SEQ ID NO:11 by substitution, insertion or deletion of up to 10% of the amino acids, and having the enzymatic activity of a GMP synthetase.

18. A protein as claimed in claim 17, which is no longer subject to feedback inhibition by secondary products of metabolic pathways starting from products of the enzyme.

19. A protein as claimed in claim 17, which is no longer inhibited by intermediates of purine biosynthesis, in particular by purine bases, purine nucleosides, purine nucleotide 5'-monophosphates or purine nucleotide 5'-diphosphates or purine nucleotide 5'-triphosphates.

20. A nucleic acid sequence coding for a protein as claimed in claim 17.

21. The use of one or more of the nucleic acid sequences as claimed in the preceding claims for the genetic engineering construction of microorganisms able to produce riboflavin.

22. A process for preparing riboflavin by cultivating microorganisms which have undergone genetic modification in at least one gene of purine biosynthesis.

23. A process as claimed in claim 22, wherein the microorganism is a bacterium of the genus Bacillus or Corynebacterium.

24. A process as claimed in claim 22, wherein the microorganism is a eukaryotic microorganism.

25. A process as claimed in claim 24, wherein the microorganism is Ashbya gossypii.

26. A process as claimed in claim 24, wherein the microorganism is a yeast.

27. A process as claimed in claim 22, wherein the microorganism is a yeast of the genus Candida, Saccharomyces or Pichia.

28. A process as claimed in claim 22, wherein the modification consists of at least one additional copy of at least one of the nucleic acid sequences as claimed in claim 5, 12, 16 or 20.

29. A process as claimed in claim 22, wherein the genetic modification generates a gene coding for a protein as claimed in claim 1, 6, 13 or 17.

Genes of purine biosynthesis from Ashbya gossypii and the use thereof in microbial riboflavin synthesis

Abstract

Genes of purine biosynthesis from Ashbya gossypii are used in microbial riboflavin synthesis.